Agrichemical Mixtures in Drinking Water and Health Outcomes in Nebraska

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July 10, 2019

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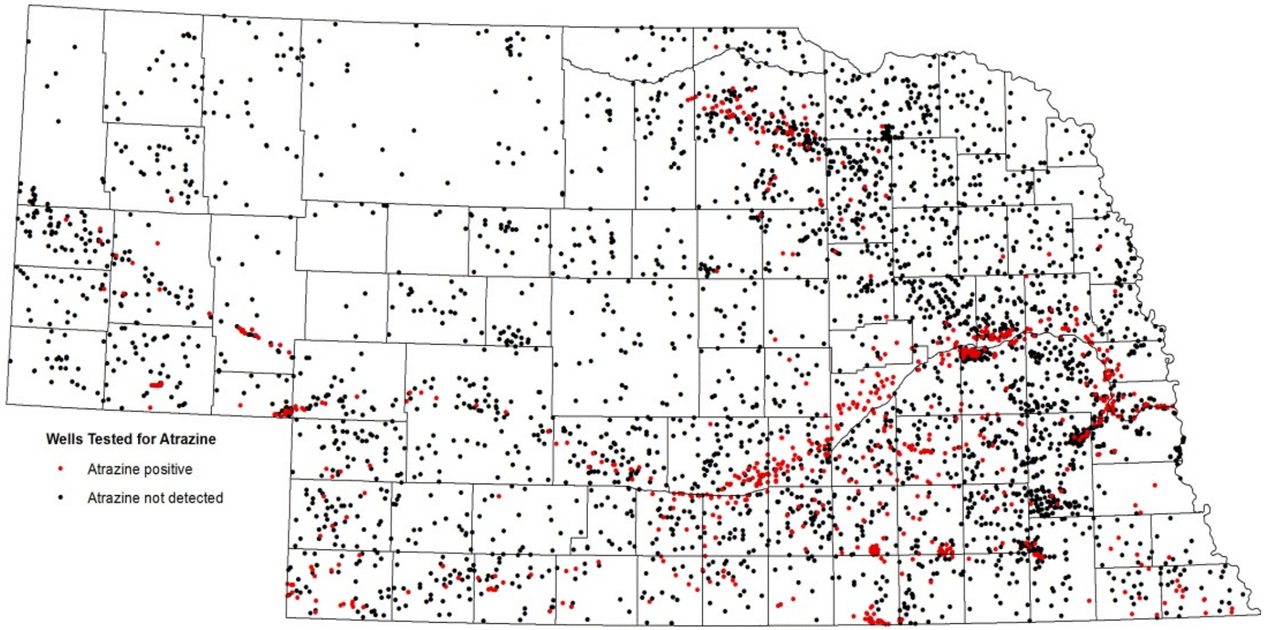
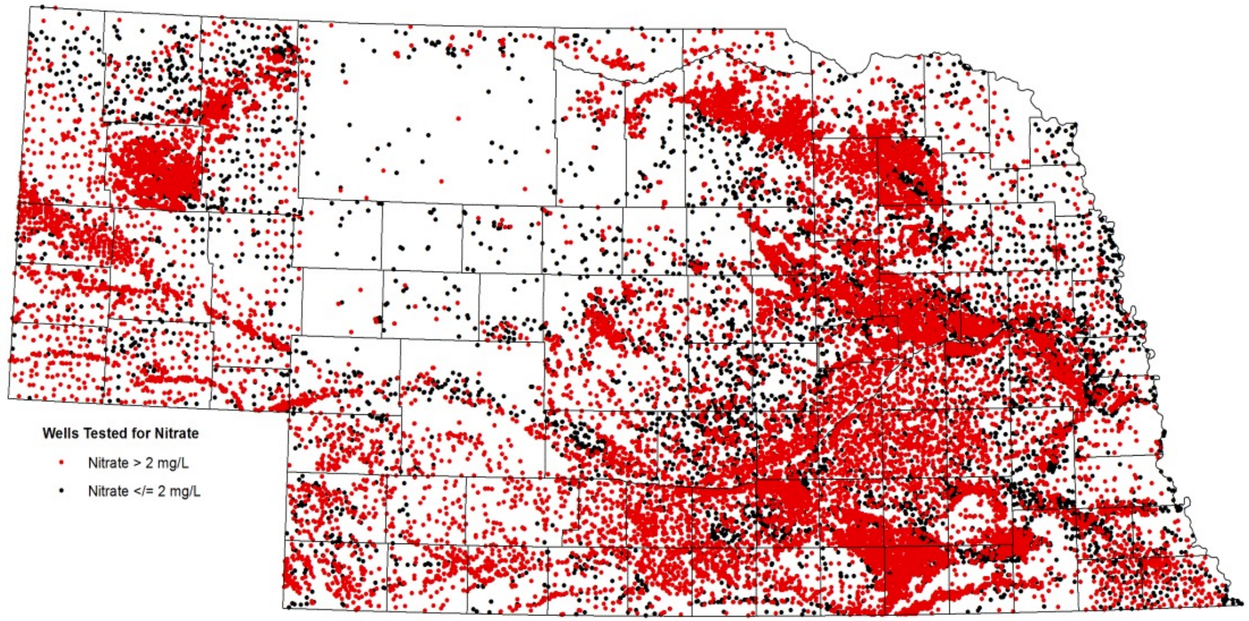
Nebraska: A model for examining health outcomes associated with exposure to agrichemical mixtures in drinking water

Atrazine and nitrate are the two most prevalent drinking water contaminants in Nebraska.
Does exposure increase risk of adverse health outcomes?



Wells sampled for **atrazine** (1977-2014)

916 positive of 4311 wells sampled



Wells sampled for **nitrate** (1977-2014)

18,843 positive (> 2 mg/L) of 25,811 wells sampled

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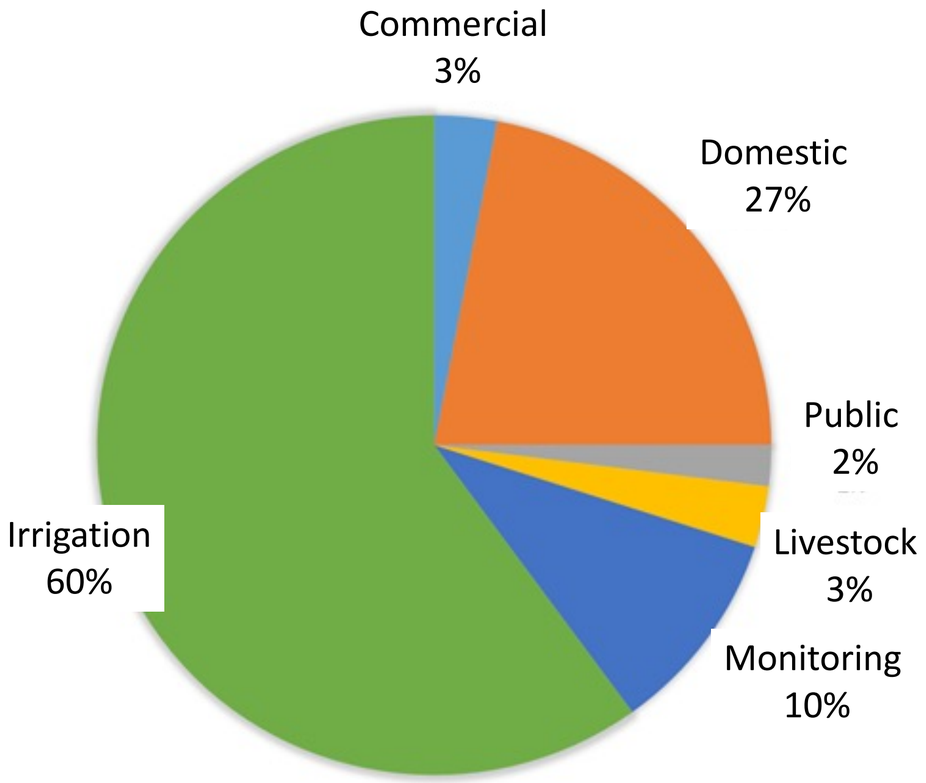
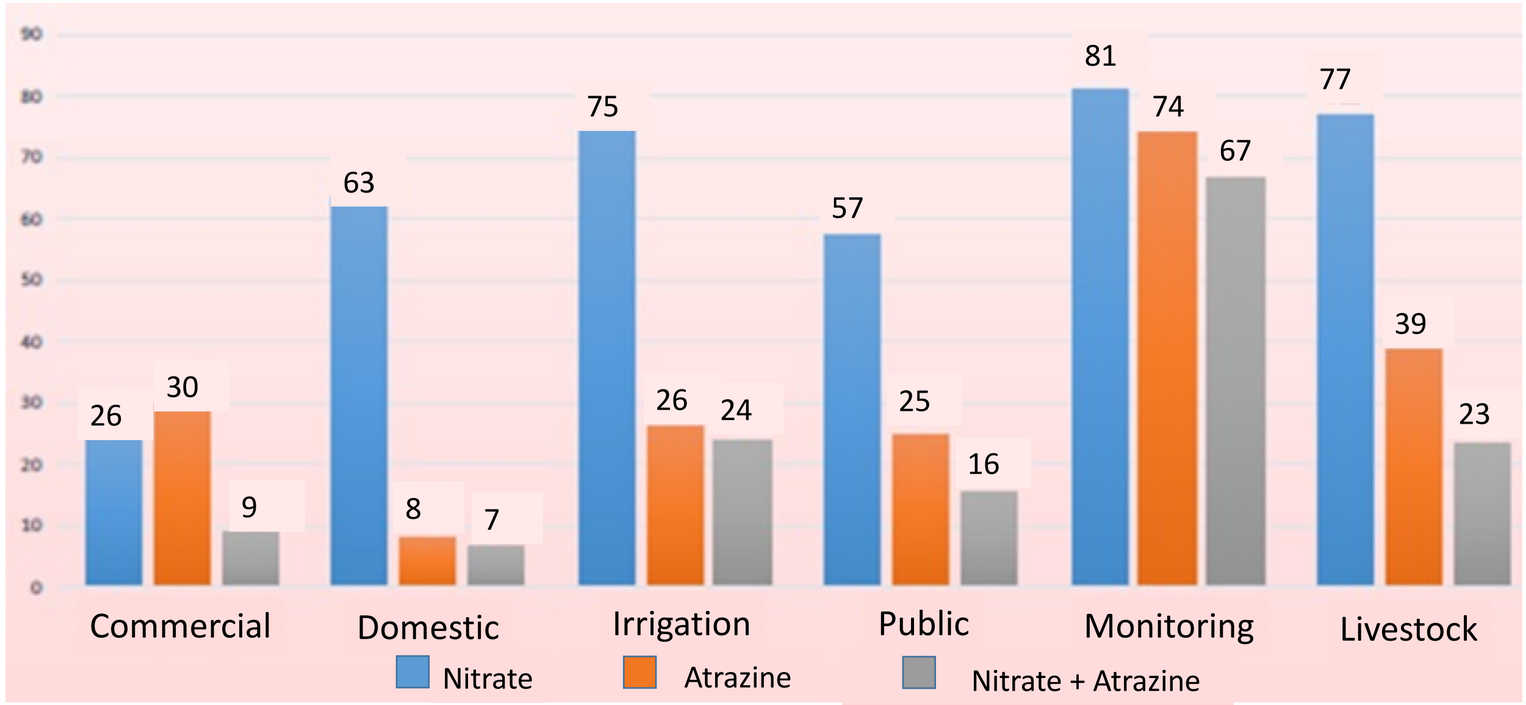
Source: Quality-Assessed Agrichemical Contaminant Database for Nebraska Groundwater (queried Fall 2015)

Agrichemical contaminated groundwater in Nebraska



Well types sampled (1977-2014)



Percent wells positive for nitrate, atrazine and combination by well type (1977-2014)

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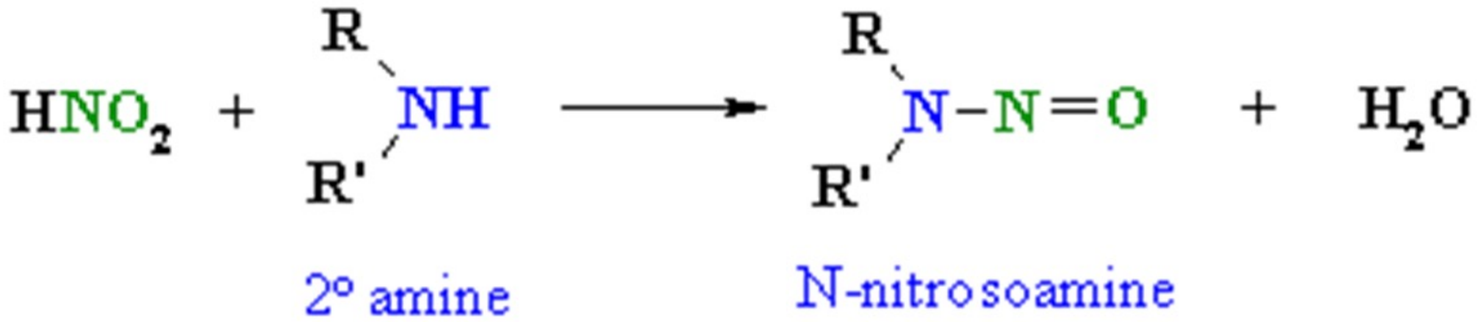
Presented at Midwest Rural Agricultural Safety and Health (MRASH) Conference November 28, 2018 by Moses New-Aaron, MPH

Adverse health outcomes from exposure to nitrate and atrazine in drinking water - is it plausible?



- *N*-nitrosoatrazine (NNAT) easily forms at pH similar to human stomach

  4:1 ratio nitrite: atrazine.

- Many nitrosamines are carcinogenic/teratogenic in animal models.
  - –NNAT → chromosomal aberrations in human lymphocytes at doses 1000 X lower than nitrate or atrazine.
- **Hypothesis – Exposure to the mixture is more toxic than exposure to either contaminant alone.**

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Atrazine and nitrate in public drinking water supplies associated with non-Hodgkin lymphoma in Nebraska

Case Control Study 1999-2002 (Chiu et al. 2005)

- 392 cases and 530 controls
  - *Demographics, health history*
  - *Primary source of drinking water*
  - *Residential history*
    - *Address for most recent residence only*
- Water Data Collected (1968-1998)
  - *Excluded subjects using domestic well as primary drinking water source*
    - *140 cases and 192 controls*
  - *Public water system (PWS) managers and NE DHHS*
    - *Concentrations for nitrate, atrazine, metolachlor, methoxychlor, 2,4-D, simazine and alachlor*
      - *Month and year of test result*
      - *Well contribution (gallons/year)*

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Atrazine and nitrate in public drinking water supplies associated with non-Hodgkin lymphoma in Nebraska

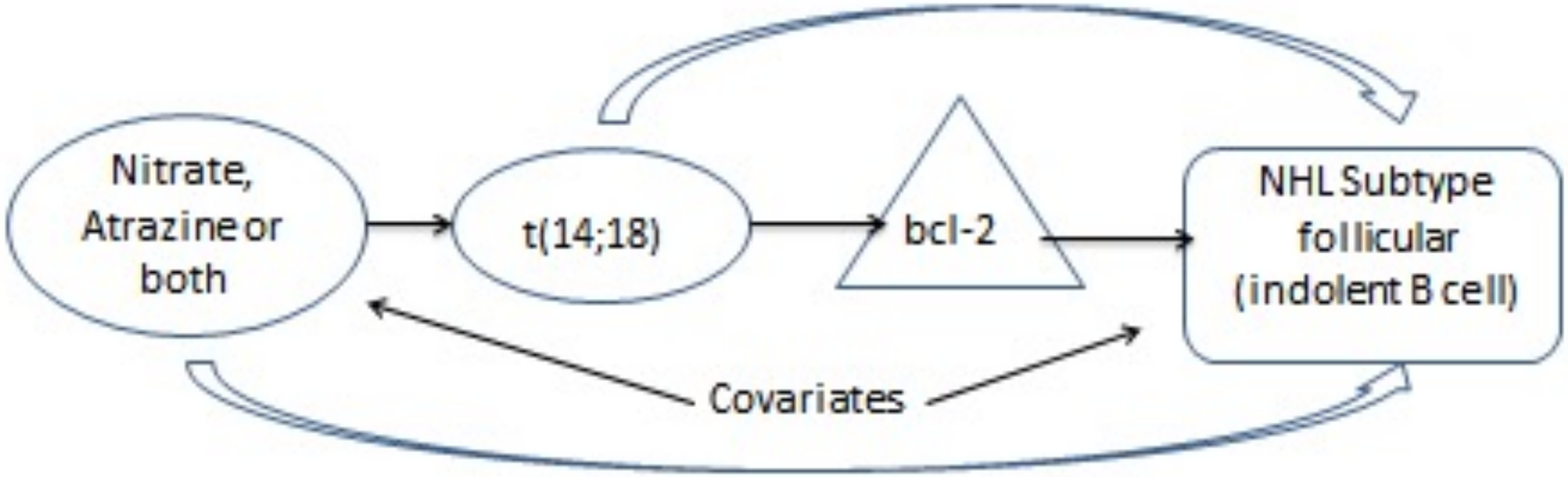
	Odds Ratio	Significance	95% CI
Nitrate	0.57	0.089	0.3-1.09
Atrazine	0.96	0.84	0.66-1.4
Atz/nitrate-NHL	**2.5**	0.047	1.01-6.16
Atz/nitrate-In.NHL	**3.47**	0.044	1.04-11.51

- NHL risk - 2.5 times higher for subjects exposed to nitrate and atrazine in drinking water compared to subjects not exposed.
- Indolent B-cell lymphoma risk - 3.5 times higher for subjects exposed to nitrate and atrazine in drinking water compared to subjects not exposed.
- Hypothesis: Increased NHL risk due to *in vivo* formation of NNAT causing chromosomal mutations during metabolism → carcinogenesis.

Rhoades MG, Meza JM, Beseler CL, Shea PJ, Kahle A, Vose JM, Eskridge KM, Spalding RF.
Environmental Health Insights 2013:7 15-27

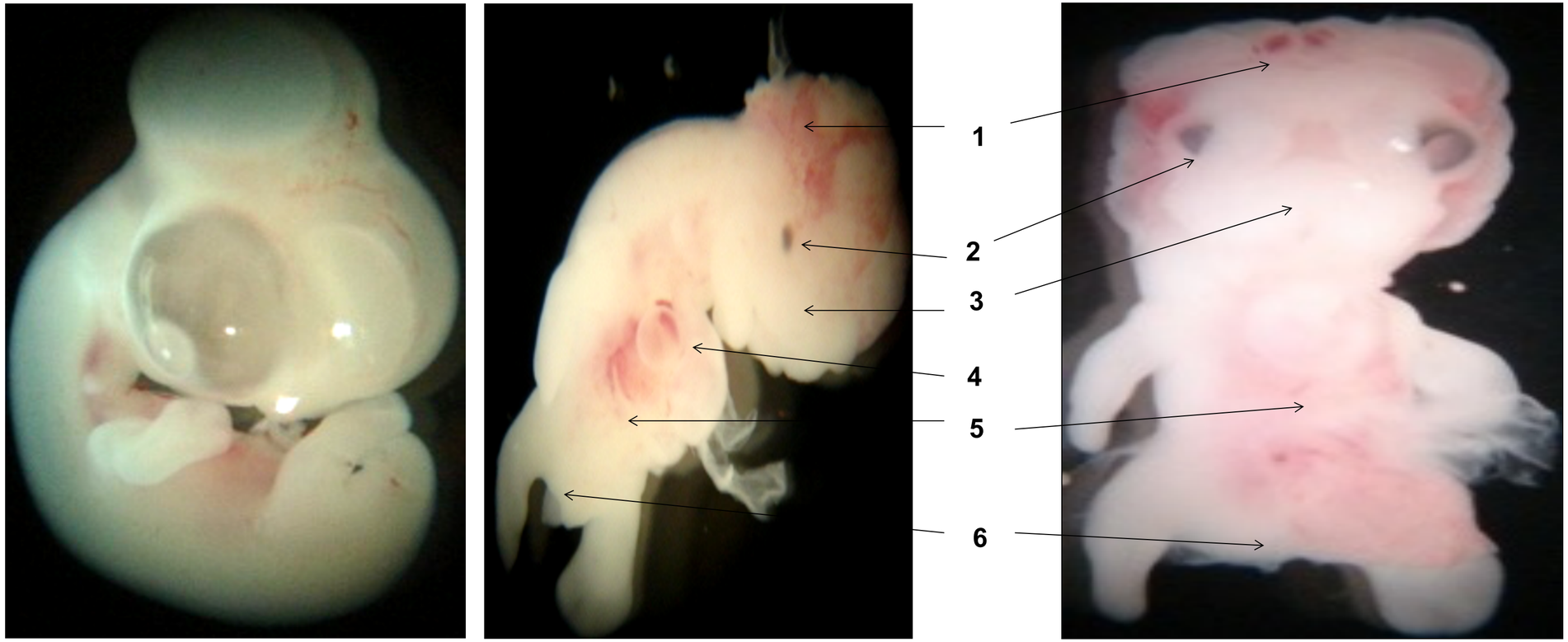
Next Steps

Are individuals exposed to nitrate and atrazine in drinking water at higher risk for developing t(14;18)-positive follicular NHL compared to t(14;18)-negative individuals or controls?



1.Reference model: NO3+ATZ → follicular NHL
2.NO3+ATZ → t(14;18) positive → follicular NHL
3.NO3+ATZ → t(14;18) positive → bcl-2 → follicular NHL
4.t(14;18) → NO3+ATZ → follicular NHL
5.t(14;18) → NO3+ATZ → bcl-2 → follicular NHL

NNAT and Avian Embryo Development



Normal 5 day **NNAT 0.46 µg** **NNAT 3.63 µg**

1. **Neural tube defect (8%)** - occurs when neural tube fails to close
2. **Microphthalmia (11%)** - abnormally small eye
3. **Craniofacial hypoplasia (11%)** - tissue deficiency or agenesis (failure of organ to develop during embryo development)
4. **Heart defects (24%)** - **Ectopic heart** displacement of heart outside thoracic cavity
5. **Gastroschisis (24%)** - intestines and other organs develop outside abdomen
6. **Caudal regression (19%)** - abnormal development of lower spine

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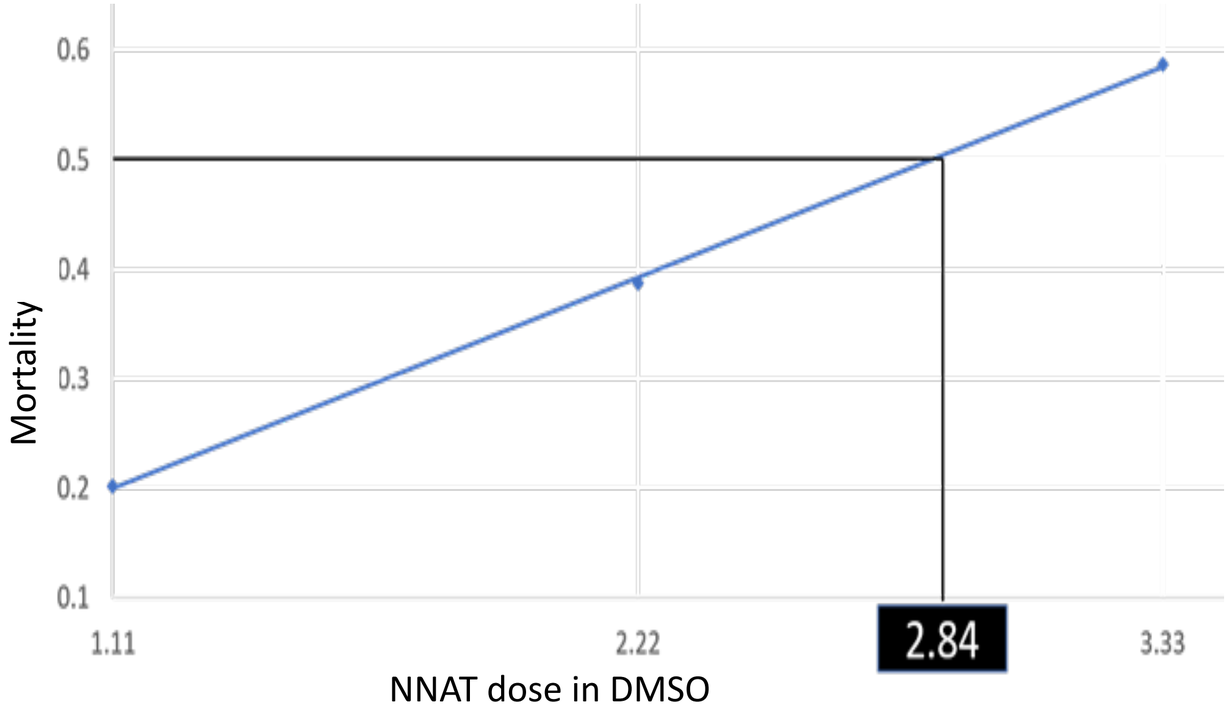
Embryo Mortality

Treatment	Mortality Probability (%)
Blank	7
DMSO	23
NNAT 1 µmol	27
NNAT 2 µmol	41
NNAT 3 µmol	58
Water	7

As NNAT dose increases, incidence of embryo death increases.

Chi-square test for mortality (p-value < 0.0001)



Lethal dose for 50% (LD50) of test animals

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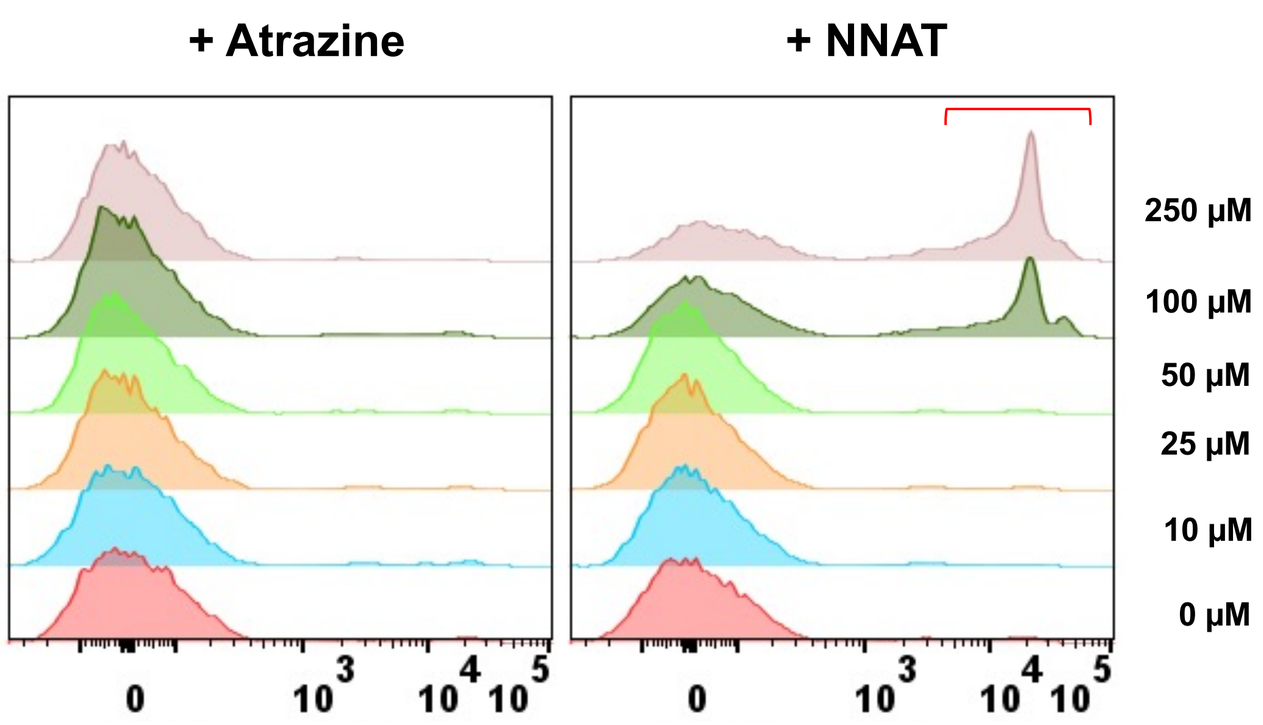
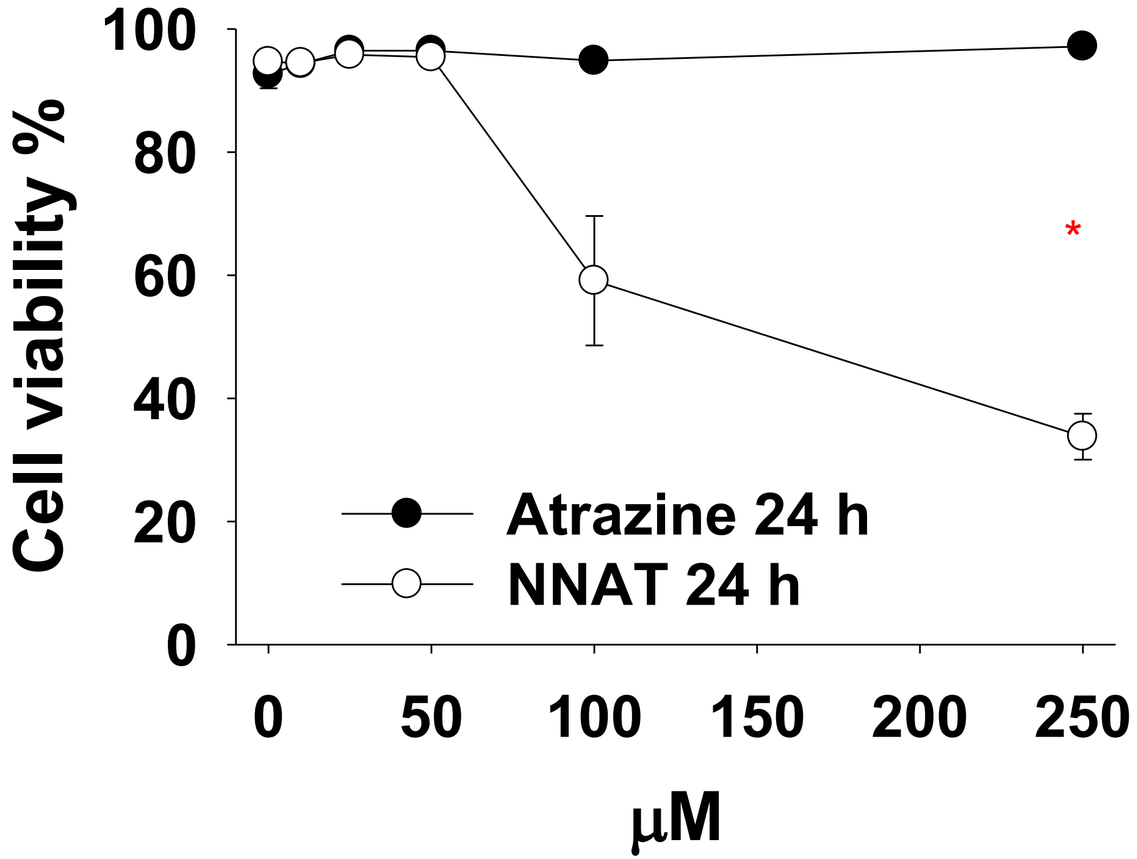
Human umbilical vein endothelial cell (HUVEC)
24° Treatment



Cell death (Propidium Iodide Uptake)



* p<0.05 One-Way ANOVA on Ranks, Tukey Test for Multiple Comparisons

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Next Steps

- Mechanistic pathway
  - *Cytochrome P450*
    - *Adult chicken liver*
    - *Whole embryos (8 day)*
  - *Nitric oxide synthase*
    - *HUVEC*
- Window of susceptibility
- Lifespan feeding study
  - *Measure stress*
    - *Hatchability*
    - *Egg fertility*
    - *Stunting of offspring*

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Birth defects in Nebraska

- National rate: Birth defects affect about 3.3% of all live births
- Nebraska rate 2005-2014: 5.8%
- 600-1200 reported birth defect cases per year
    - Cardiovascular (500+)
    - Central nervous system (100+)
    - Gastrointestinal (250+)
    - Genitourinary (550+)
    - Musculoskeletal (250+)

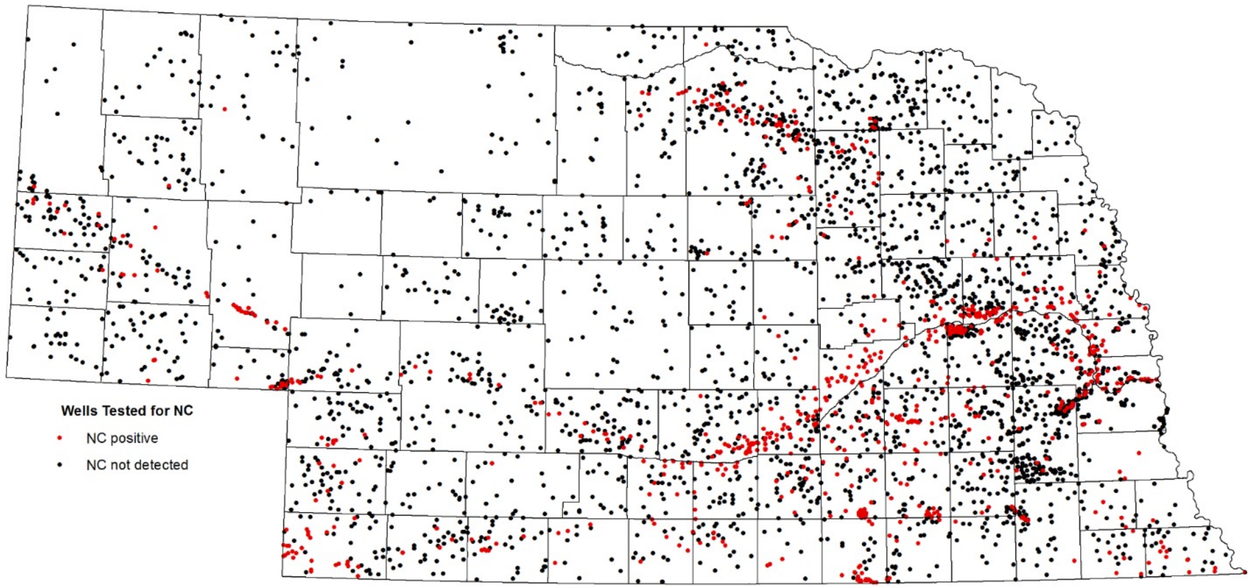
Nitrosatable agrichemicals detected in Nebraska groundwater wells

Metolachlor ESA* 70% (28; 107)	Deethyl-cyanazine 67% (4; 12)	Alachlor ESA* 52% (28; 107)	**Deisopropyl-atrazine 37% (82; 1,927)**	**Deethyl-atrazine 25% (83; 2,081)**	Alachlor ESA* 2°Amide 24% (23; 69)
Propazine 17% (66; 1,988)	Alachlor OA** 16% (19; 56)	Metolachlor OA** 12% (28; 107)	Acetochlor ESA* 11% (28; 107)	Hydroxyalachlor 11% (5; 9)	Hydroxy-simazine 8% (4; 12)
Acetochlor OA** 7% (28; 107)	Alachlor 6% (93; 4,454)	Prometon 4% (87; 2,291)	Acetochlor 3% (77; 1,591)	Bromacil 3% (74; 595)	Simazine 3% (87; 2,430)
Propachlor 2.7% (85; 2,223)	Cyanazine 2% (93; 4,451)	Metolachlor 2% (93; 4,300)	Trifluralin <1% (93; 4,186)	Ametryn <1% (62; 795)	Metribuzin <1% (93; 4,345)
Prometryn <1% (63; 797)	Butylate <1% (93; 4,300)	S-Ethyl-N,N-dipropylthiocarbamate <1% (77; 1,842)	Pendimethalin <1% (75; 1,458)		

Percentage of positive wells tested for nitrate + NC
(# counties; # wells)
1,518 of 4,495 wells sampled were positive for nitrate + NC (~34%)

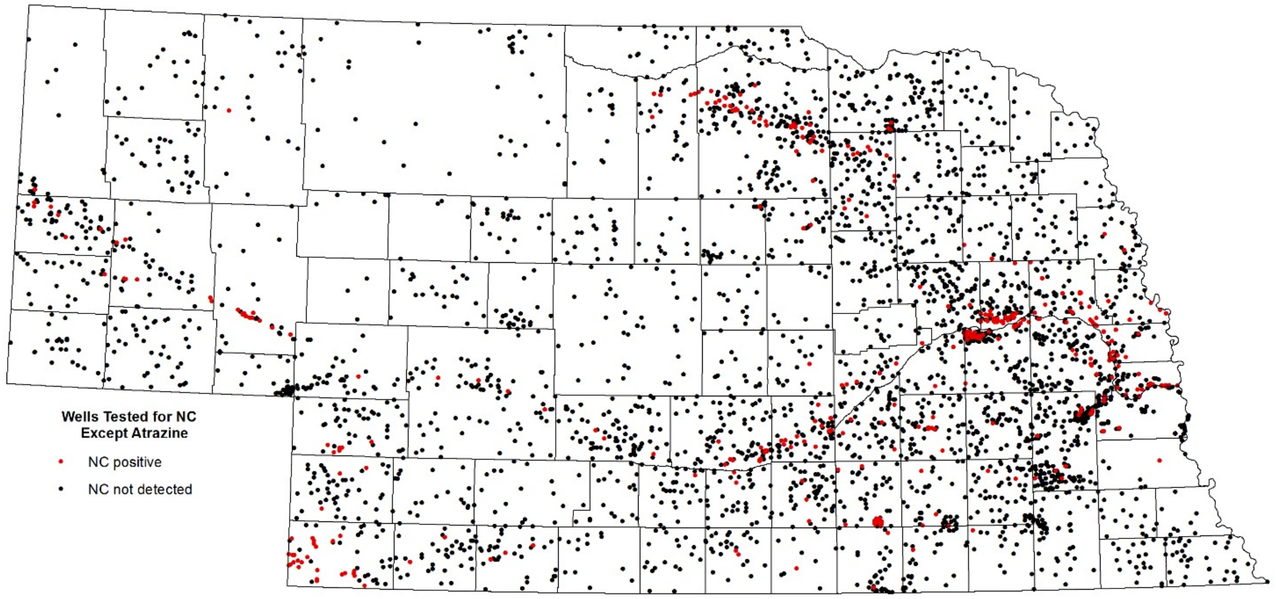
*ethanesulfonic acid
**oxanilic acid

Nitrosatable agrichemicals detected in Nebraska groundwater wells



Wells sampled for any NC (1977-2014)

1122 positive of 4736 wells sampled



Wells sampled for any NC except atrazine (1977-2014)

853 positive of 4736 wells sampled

Source: Quality-Assessed Agrichemical Contaminant Database for Nebraska Groundwater (queried Fall 2015)

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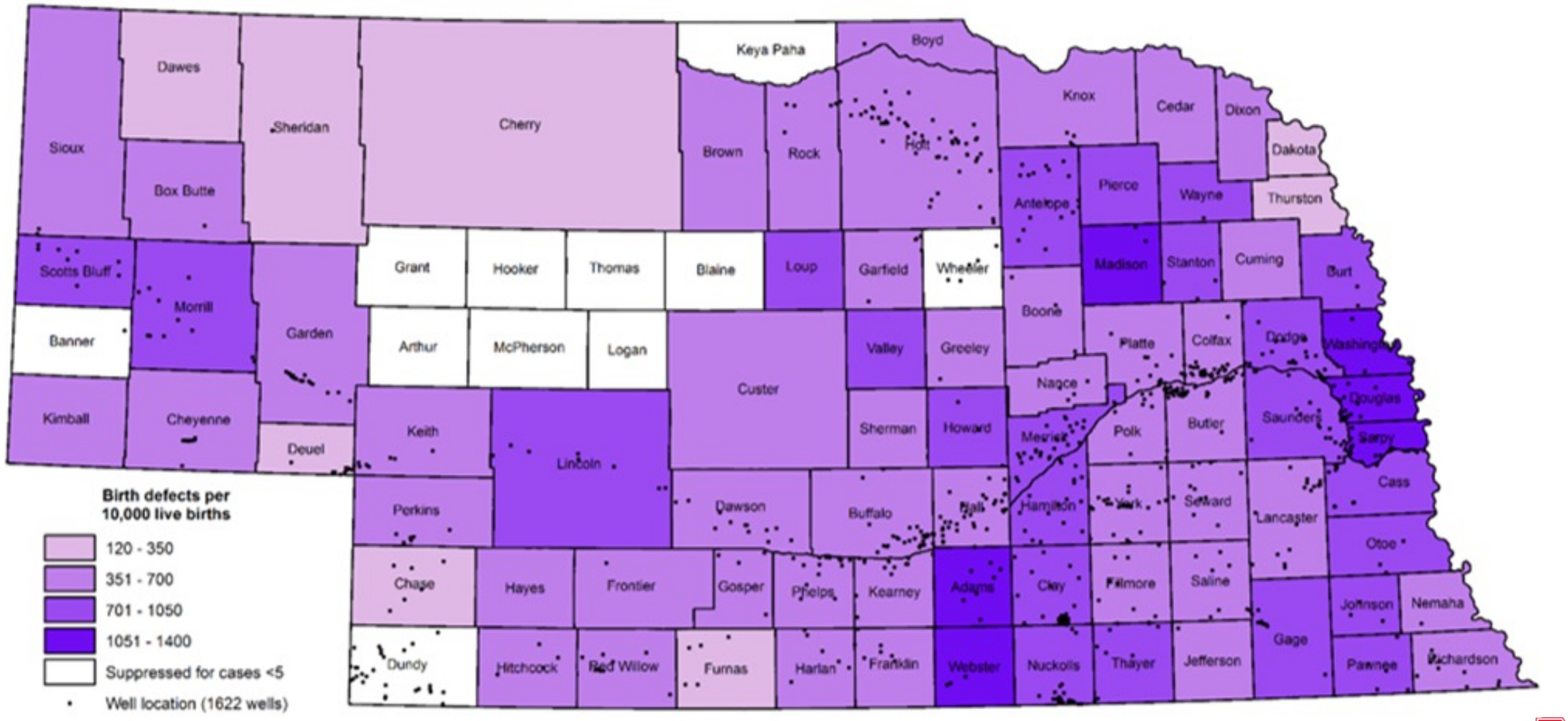
Nebraska birth defect rates by county and wells positive for nitrate + nitrosatable agrichemical



Birth defect rates 2005-2014. Source: Nebraska Department of Health and Human Services

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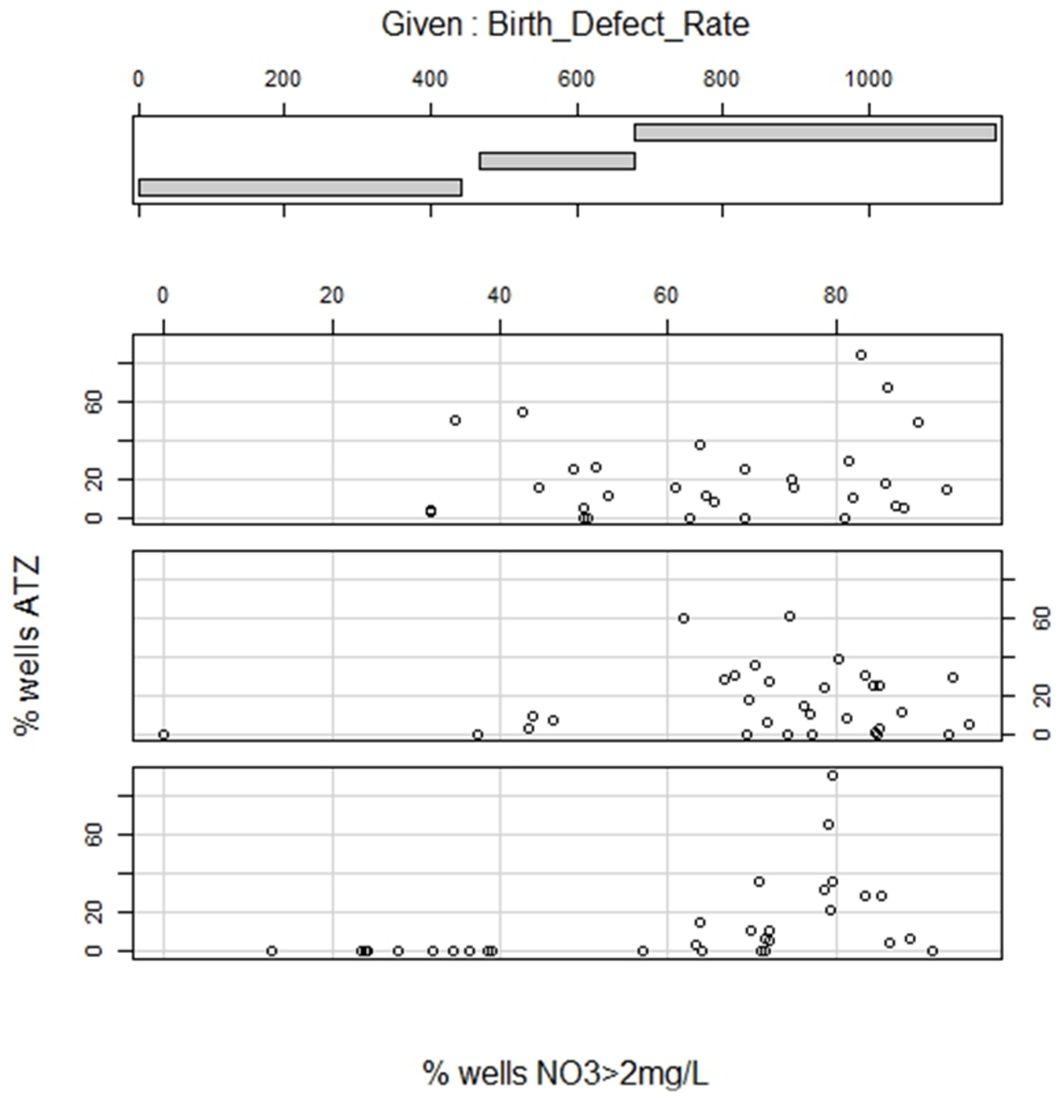
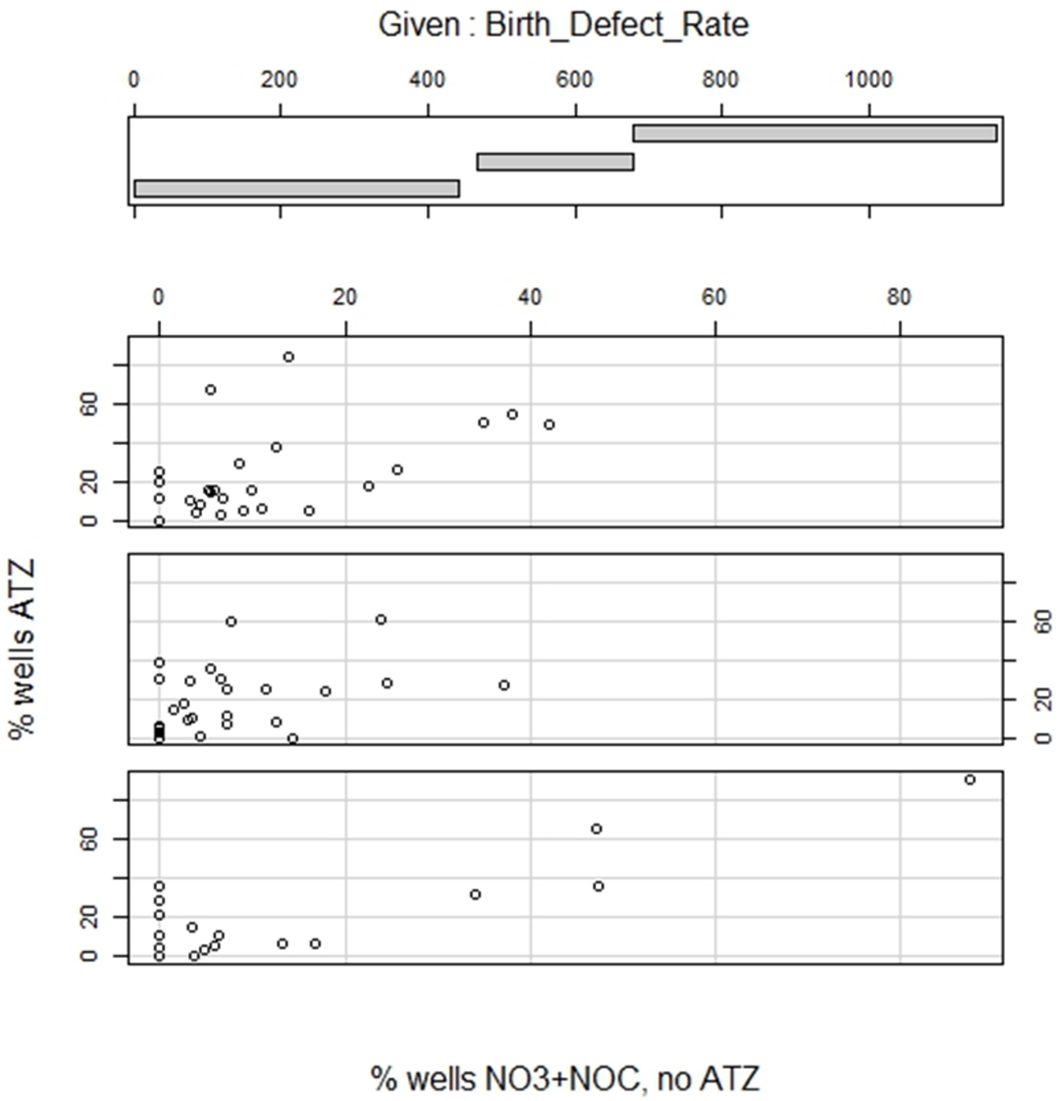
Regression analysis results with the agrichemical of interest predicting birth defect rates in 93 Nebraska counties.

Percentage of Wells Testing Positive	Effect Size	Std. Error	p-value	95% Confidence Interval
Atrazine	2.74	1.37	0.05	0.06-5.42
Nitrate greater than 0 mg/L	-3.71	2.44	0.13	-8.49-1.07
Nitrate greater than 2 mg/L	1.06	1.32	0.42	-1.53-3.65
Nitrate + NC	3.02	1.43	0.04	0.22-5.82
Nitrate + atrazine	2.83	1.49	0.06	-0.09-5.75
Nitrate + NC except atrazine	2.28	1.97	0.25	-1.58-6.14





Co-plots of contaminants conditional on being in one of three birth defect groups where each birth defect group represents 31 counties.

Evidence from observational study

Association between domestic wells and other well types

Percent wells positive for nitrate		Correlation coefficient (p-value)
Domestic	Livestock	0.77 (<0.001)
	Public	0.59 (<0.001)
	Commercial	0.61 (0.03)
Percent wells positive for atrazine		**Correlation coefficient (p-value)**
Domestic	Public	0.32 (0.04)
	Monitoring	0.55 (<0.001)
	Irrigation	0.45 (<0.001)

Linear regression between birth defect rates and percent agrichemical-positive wells

Agrichemical(s)*	Slope	p value
Any NCs	3.12	0.02
Only Parent (P) NCs	2.92	0.03
Only Degradate (D) NCs	2.16	0.26
Nitrate	-4.33	0.07
Atrazine	3.03	0.03
Nitrate D	-2.71	0.14
Atrazine D	5.7	0.02
Nitrate P	-6.37	0.02
Atrazine P	1.87	0.05
Nitrate P+D	-2.45	0.19
Atrazine P+D	6.44	0.002
Nitrate+Atrazine D	5.73	0.03
Nitrate+Atrazine P+D	6.9	0.005

***=Percent wells positive for NCs=Nitrosatable compounds**

P=Public wells D=Domestic wells

Limitations

NHL study

- Missing data
- Exposure is binary
- PWS water data only
- Pesticide exposure history
- Occupational history
- Diet history
- Other drinking water contaminants

Embryotoxicity study

- No dose response
- Chick embryo model
- Window of susceptibility
- Mechanism for toxicity

Birth Defects study

- Ecological study
- Other risk factors
- Agrichemical mixtures

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ONGOING RESEARCH - Pilot/feasibility case-control study

- Nebraska women (n=40)
  - 5 each water system
- Questionnaire
  - demographics/health/residential history
- Water sample
  - Nitrate/pesticide analysis
  - Age dating
- Saliva sample
  - Salivary nitrate→nitrosation potential
- Blood sample
  - Gene x Environment Interactions
    - CYP2E1 and NQO1 genes
    - Chromosomal aberrations
- Participant Perception
  - Barriers/motivation to participate

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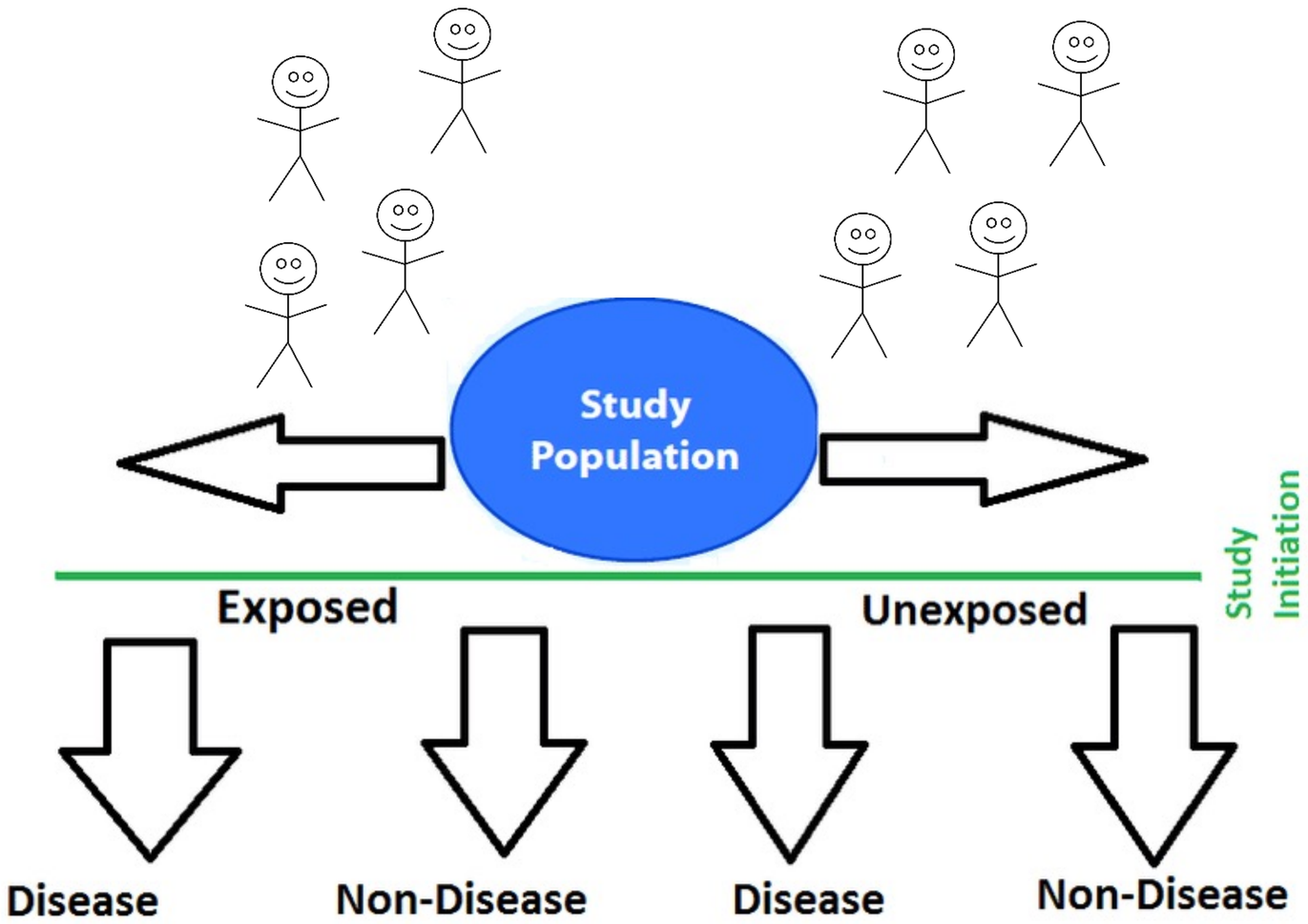
What's next?

- Nebraska may be a good venue to conduct a cohort study to better understand the human health impacts of agrichemicals in drinking water.
- If there is an association, we can develop preventive measures.



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Challenges

- Increase public understanding of issues and related research
  - Without causing alarm
  - Importance of participation in this type of study
  - Translating research findings to lay community
- Researcher, community and stakeholder bridge
  - Partnerships
  - Public perception
  - Adapting methodology to increase participation
  - Engagement and collaboration

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Acknowledgements

NHL Study Participants

NHL Case Control Data

- Brian Chiu, PhD

Water Data

- Andy Kahle
- Water Operators
- Colleen Steele

Mapping

- Les Howard

Funding

- University of Nebraska Center for Environmental Toxicology
- NU Research Council
- NU Collaboration Initiative
- Robert B. Daugherty Water for Food Global Institute

Students

- Moses New Aaron, Jeffrey Wallman
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  - Pat Shea, PhD
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Questions?

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